Research Article

Characterization of high molecular weight coffee fractions and their fermentation by human intestinal microbiota

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To investigate the structure and fermentability of high M_r components of coffee brews by human gut bacteria Arabica coffee samples of three different degrees of roast (light, medium, and dark) were used for drip brew preparations and fractionation by ultrafiltration with different M_r cut-offs. Total carbohydrates of the fractions ranged from 28.6 g/100 g to 56.7 g/100 g. Galactomannans and arabinogalactans were the main polysaccharides and made up between one-fourth and one-half of the respective coffee fraction. After 24 h of incubation with a human fecal suspension the polysaccharides of all fractions were extensively degraded. A decrease in the absorbance values at 405 and 280 nm, respectively, indicated that also chemically noncharacterized UV-active components such as Maillard reaction products, had been partially degraded or modified by the human gut bacteria. The remainder after 24 h of fermentation still showed antioxidant activity. Bacterial cells belonging to the *Bacteroides–Prevotella* group increased 2- to 40-fold during fermentation depending on the M_r range of the fraction and the degree of roast. The production of high amounts of acetate and propionate is in accordance with a role of these bacteria in the degradation of high M_r components from coffee.

Keywords: *Bacteroides–Prevotella* group / Degree of roast / Galactomannans / High molecular weight coffee fractions / Maillard reaction products

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1 Introduction

High M_r components (polysaccharides, proteins, and Maillard reaction products) make up more than 30% of the dry matter of coffee brews. Galactomannans and type II arabinogalactans were shown to be major components of the coffee dietary fiber fraction, which is effectively metabolized by human intestinal bacteria *in vitro* [1]. Since high M_r melanoidins are presumably not absorbed in the small intestine [2], they serve as potential substrates for the colonic microbiota and influence the microbial composition [3]. Because reactive oxygen species are supposed to play a role in colon carcinogenesis [4], the antioxidant properties of melanoidins reaching the colon are also an important factor.

The experiments presented here build upon our previous study in which we investigated the degradation of coffee dietary fiber prepared from medium roasted coffee [1]. The objective of the present study was to investigate the *in vitro* fermentability of different high M_r fractions (3–10, 10–50, 50–100, and >100 kDa) prepared by ultrafiltration from coffee brews of three different degrees of roast (light, medium, and dark). Contrary to our previous experiments the high M_r components were fractionated according to their M_r with the objective to obtain different types of molecules. Apart from polysaccharide degradation and structural changes in the carbohydrate constituents we analyzed changes in the UV-absorbing, brown products, which are assumed to have their origin in the Maillard reaction by monitoring the color as well as the reducing and radical scavenging properties of the fermented fractions. In addition, microbial metabolites such as SCFA were measured and changes in the microbial composition were analyzed by

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Abbreviations: **ABTS,** 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); **Cs,** Sorensen's pairwise similarity coefficient; **DGGE,** denaturing gradient gel electrophoresis; **FISH,** fluorescence *in situ* hybridization; **MWCO,** M_r cut-off; **RE,** reduction equivalents; **TEAC,** Trolox equivalent antioxidant capacity

fluorescence *in situ* hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE).

2 Materials and methods

2.1 General

All chemicals and solvents were of analytical grade. Reagents for FISH and PCR-DGGE were of molecular biological grade. The probes used for FISH were from Thermo Electron (Ulm, Germany) and the primers used for PCR-DGGE were from MWG (Martinsried, Germany). Lysozyme was purchased from Roche (Mannheim, Germany) with a purity of 95–100% and an activity of >23 000 U/mg according to Shugar. All other chemicals were from Merck (Darmstadt, Germany) or Sigma–Aldrich (Schnelldorf, Germany).

2.2 Coffee samples, brewing procedure, and preparation of high M_r fractions

Arabica coffee samples (origin Colombia) of three different roasting degrees (light, medium, and dark roasted) were kindly provided by Tchibo Manufacturing KG (Hamburg, Germany). Roasted coffee samples were ground with an average particle size of 450 µm representing a powder typically used for drip-brewed coffees. Drip brews were prepared using a customary coffee maker (brewing temperature, 96–99°C), no. 4 sized cone paper filters, 50 g of coffee powder, and 1 L of water, resulting in approximately 900 mL of coffee brews.

Cold-water insoluble compounds were removed after freeze drying of the coffee brew, re-solubilization in water and centrifugation at 2000 × *g* and 20°C. The supernatant was membrane filtered (0.45 µm pore size) and used for preparation of high M_r fractions by ultrafiltration. Tangential flow ultrafiltration units with a polyethersulfone membrane and M_r cut-offs (MWCO) of 100, 50, 10, and 3 kDa (Vivaflow 50, Sartorius AG, Goettingen, Germany) and a flexible-tube pump (Masterflex L/S pump drive 7554-85 and easy-load pump head 7518-00, Cole–Parmer Instrument, Vernon Hills, USA) were used. Four high M_r coffee fractions (>100, 50–100, 10–50, and 3–10 kDa) were obtained by stepwise ultrafiltration using each filtrate for the next ultrafiltration step. After freeze-drying and homogenizing in a mortar the retentates were weighed and used for chemical characterization and fermentation studies.

2.3 Fecal samples and culture conditions

Fecal samples were collected from one healthy volunteer who had no previous history of gastrointestinal disorders and had not undergone antibiotic therapy within six months prior to the study. Fresh fecal samples were prepared as described elsewhere [1]. The OD at 600 nm was adjusted to a final value of ~1.7. Fermentations were carried out in 16 mL glass tubes containing 9 mL basal liquid medium [1]. The final concentration of the coffee fraction or glucose solution was 1.8 mg/mL. Aliquots (1.5 mL) were taken after 0, 6, 12, and 24 h of incubation and analyzed as described below. In general, fermentations were carried out in triplicate. However, due to the low yields of some M_r fractions (Table 1) their amounts were not sufficient to perform fermentations in triplicate.

An additional fermentation experiment was performed using the M_r fractions from medium roasted coffee. This particular fermentation experiment was performed with a total volume of 100 mL in 250 mL glass vessels to obtain sufficient material for the microbial population analysis by DGGE and structural characterization of the coffee fractions.

2.4 Determination of OD, pH, and SCFA

Batch culture (0.5 mL) was centrifuged for 5 min at 14 000 × *g* and 4°C, the pellet was resuspended in PBS (1 mL) and the resulting OD was determined at 600 nm with a Beckman DU-640 spectrophotometer (Beckman Instruments, Fullerton, USA). The pH was determined by using a MultiCal pH meter (WTW, Weilheim, Germany). Gas chromatographic SCFA determination was performed as described earlier [1].

2.5 Fractionation of fermented samples and preparation of fermented samples for chemical analysis

Whereas unfermented high M_r coffee fractions were analyzed as prepared, fermented coffee samples were centrifuged for 6 min at 4°C and 10 000 × *g* to remove bacterial cells and the supernatants were subsequently subjected to chemical analysis.

Using centrifugal ultrafiltration (MWCO 1 kDa; Microsep®, Pall Corporation, East Hills, NY, USA), aliquots (corresponding to 5 mg of unfermented sample) of the cell-free fermented samples were further fractionated in low and high M_r material (<1 and >1 kDa, respectively). Retentates were washed three times with water. After freeze-drying retentates and filtrates were further analyzed. Analysis of retentates included the determination of reducing and antioxidant activity *in vitro* and absorbance measurement at 405 and 280 nm as described below. Filtrates were only analyzed spectrophotometrically at 405 and 280 nm.

2.6 Determination of total carbohydrates and monosaccharide residues

The total carbohydrate content, calculated as the sum of anhydrosugars, was determined using the phenol–sulfuric acid method [5] as recently described [1]. Neutral sugars

Table 1. Cy3 labeled 16S rRNA targeting oligonucleotide probes used for FISH

Probe	Sequence (5′–3′)	OPD code	Target organism	Ref.
EUB mix			Total bacteria	[30]
EUB 338	GCTGCCTCCCGTAGGAGT	S-D-Bact-0338-a-A-18	–	[31]
EUB 785	CTACCAGGGTATCTAATCC	S-D-Bact-0785-a-A-19	–	[32]
EUB 927	ACCGCTTGTGCGGGCCC	S-D-Bact-0927-a-A-17	–	[33]
EUB 1055	CACGAGCTGACGACAGCCAT	S-D-Bact-1055-a-A-20	–	[32]
EUB 1088	GCTCGTTGCGGGACTTAACC	S-D-Bact-1088-a-A-20	–	[32]
Erec 482	GCTTCTTAGTCARGTACCG	S-*-Erec-0482-a-A-19	*C. coccoides–E. rectale* group	[34]
Bac 303	CCAATGTGGGGGACCTT	S-*-Bacto-0303-a-A-17	*Bacteroides–Prevotella* group	[35]
Bif 164	CATCCGGCATTACCACCC	S-G-Bif-0164-a-A-18	Bifidobacteria	[36]

were analyzed by GC-FID as their alditol acetates after being released by modified Saeman hydrolysis (pre-hydrolysis with 12 M H_2SO_4 for 5 min at room temperature, hydrolysis using 2 M H_2SO_4 for 60 min at 100°C) [6]. GC conditions were described elsewhere [1].

2.7 Structural characterization of carbohydrates

Carbohydrate structural characteristics were determined by methylation analysis as described before [7, 1]. In brief, dry samples were activated with powdered NaOH in dimethylsulfoxide and methylated with CH_3I. After remethylation the material was hydrolyzed with TFA and partially methylated monosaccharides were reduced with $NaBD_4$. Following acetylation with acetic anhydride and 1-methylimidazole, identification and quantification of partially methylated alditol acetates was carried out by GC-MS and GC-FID as detailed elsewhere [1]. Molar response factors were used according to Sweet *et al*. [8].

2.8 Determination of absorbance at 405 and 280 nm, reducing properties and antioxidant activity in vitro

Aqueous solutions of high M_r coffee fractions (0.1 mg/mL) and fermented samples (aqueous solutions corresponding to 0.1 mg/mL of unfermented material) were measured spectrophotometrically at 280 and 405 nm. Aqueous solutions of medium roasted coffee fractions were additionally characterized by color dilution analysis according to Hofmann *et al.* [9] (starting concentration 1 mg/mL).

Reducing properties were determined colorimetrically by the ferricyanide method [10], which is based on the reduction of hexacyanoferrate (III) to hexacyanoferrate (II) in a phosphate buffered solution (pH 6.6, 0.2 M). A mixture of aqueous sample solution (1 mL, 0.1 mg/mL), phospate buffer solution (1 mL), and 1% aqueous potassium hexacyanoferrate (III) solution (1 mL) was freshly prepared in a screw-cap tube, sealed and incubated for 20 min at 50°C in the dark. After quickly cooling down the solution to room temperature, 10% aqueous trichloroacetic acid solution (1 mL), water (4 mL), and 0.1% aqueous iron (III) chloride solution (0.8 mL) were added and the absorbance was measured after 10 min at 660 nm against a reagent blank. Calibration was carried out using potassium hexacyanoferrate (II) as a standard substance. Reduction equivalents (RE values) were calculated as the amount (μmol) of hexacyanoferrate (II) produced by 1 mg of sample.

Antioxidant activity was evaluated using the leucomethylene blue methodology [11] as described by Lindenmeier *et al*. [12] measuring the potential of a sample to inhibit the formation of linoleic acid hydroperoxides in a phosphate buffered solution (pH 6.75). Each sample was analyzed in triplicate. In a second approach, the antioxidant capacity was evaluated using the Trolox equivalent antioxidant capacity (TEAC) assay, based on the decolorization of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation [13]. An aqueous solution containing ABTS (7.0 mM) and potassium persulfate (2.45 mM) was allowed to stand in the dark at room temperature for 12–16 h generating $ABTS^{6+}$. Then the solution was diluted with PBS (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm (equilibrated at 30°C). To measure $ABTS^{6+}$ decolorization, diluted $ABTS^{6+}$ solution (1.0 mL) was pipetted into disposable cuvettes (1 cm path length) containing sample solution (10 μL in PBS) and exactly 6 min after initial mixing the absorbance was measured at 734 nm against a solvent blank. Four differently concentrated sample or standard (Trolox, 0.1–0.4 μg/mL) solutions were measured, producing a 20–80% inhibition of the blank absorbance. The percentage of inhibition was plotted as a function of the concentration (mg/mL) and Trolox equivalents (TE values) were calculated by dividing the slope of the sample plot by the slope of the plot obtained for Trolox (% inhibition *vs*. concentration in μg/mL). Each sample was analyzed in triplicate.

2.9 Fluorescence *in situ* hybridization and enumeration of bacterial cells

The oligonucleotide probes used in this study were labeled with the fluorescent dye Cy3 at the 5′-end and are listed in Table 1. Batch culture aliquots (1.0 mL) were centrifuged for 5 min at 12 000 × *g* and 4°C. The pellet was resuspended

in PBS (0.5 mL), added to paraformaldehyde (1.5 mL; 4% aqueous solution) and fixed for 4 h at 4°C. Fixed samples were stored at −80°C until further use. For microscopic sample preparation Teflon coated eight-well slides (Roth, Karlsruhe, Germany) with a well diameter of 6 mm were used. The fixed samples were homogenized for 1 min at full speed in a Gyroprep (UniEquip Laborgerätebau und Vertriebs GmbH, Martinsried, Germany) and diluted depending on the probe used. Tween solution (10 μL; 0.01%) was applied to each well and the fixed and diluted fecal sample (10 μL) was added. The suspension was allowed to air dry before being dehydrated for 3 min each in 60, 80, and 96% ethanol. In case of using probes targeting gram-positive groups of bacteria, samples were treated with lysozyme buffer (14 μL; 1 mg/mL lysozyme, 100 mM Tris-HCl, 50 mM EDTA) for 10 min at 4°C. Subsequently, the lysozyme was removed with bidistilled H_2O and the slides were air dried and dehydrated using ethanol as described above. Each well was covered with a mixture of hybridization buffer (10 μL; 0.9 M NaCl, 10 mM Tris HCl pH 7.4, 0.01% SDS) and probe solution (1 μL; 10 pmol/μL in hybridization buffer), except for Bac303, which was used at a four-fold concentration in hybridization buffer with 30% v/v formamide. The slides were kept in a moist chamber for overnight hybridization and washed in hybridization buffer for 30 min at a temperature 2°C higher than the one used for hybridization. The slides were air dried and mounted with Vectashield (Vector Labs, Peterborough, UK). Samples were counted with an Axioplan2 imaging microscope (Carl Zeiss, Oberkochen, Germany), equipped with a servo-controlled microscope stage (EM14MOT, Merzhäuser, Wetzlar, Germany), a Plan-Neofluar 40× dry and 100× oil immersion objective (Carl Zeiss, Oberkochen, Germany), and the fluorescence filter set 15 (excitation, 546 nm; emission. 590 nm) for Cy3 excitation.

2.10 DNA-isolation, PCR and DGGE for investigation of changes in the microbial composition during fermentation

Aliquots (20 mL) were centrifuged for 5 min at 10 000 × *g*. The pellet was used for DNA isolation with the InViTek DNA-Isolation Kit III (InViTek, Berlin, Germany) according to the manufacturer's instructions. The DNA was used as a template to amplify the variable regions V6–V8 of the bacterial 16S rRNA genes with primers U968-GC-f (5′-*CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG* GAA CGC GAA GAA CCT TAC-3′) and L1401-r (5′-CGG TGT GTA CAA GAC CC-3′) [14]. The G + C clamp at the 5′-end of the primer U968-GC-f is underlined. Each PCR reaction mixture of 50 μL contained 10 mM dNTPs, 4.5 mM MgCl2, 2.5 U of Taq polymerase, forward and reverse primers (10 pM each) and 1 μL of 1:10 diluted DNA solution obtained from the DNA extractions. PCR amplification was performed using a PCR thermal cycler (ThermoHybaid, Ashford, UK) as follows: initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, and touchdown primer annealing temperature decreasing linearly from 66 to 59°C within 21 cycles for 20 s, followed by 14 cycles with an annealing temperature of 59°C for 20 s, and primer extension at 72°C for 40 s. The PCR amplification was run for a total of 35 cycles followed by a final extension for 10 min at 72°C. For DGGE analysis the amount of PCR products was estimated by comparison with a low DNA mass ladder (Invitrogen, Karlruhe, Germany) on a 1% agarose gel in TAE-buffer (0.04 M Tris, 0.1 M EDTA (pH 8), 0.02 M acetic acid). Approximately 80 ng of PCR product was analyzed by DGGE with the CBS Scientific DGGE system (CBS Scientific, Del Mar, CA, USA) as described before [15] but with a 35–55% gradient of urea and formamide. DGGE gels were scanned using an imaging densitometer (BioRad, München, Germany) and analyzed with the Quantity One software version 4.3.1 (BioRad). Comparison of the DGGE band patterns was performed using the similarity matrix function of the software which calculates Sorensen's pairwise similarity coefficient (Cs) [16]. Two identical band patterns result in a Cs value of 100% whereas completely different patterns with no common band result in a Cs value of 0%.

3 Results and discussion

3.1 Yields and characteristics of high M_r coffee fractions

Approximately 2 g cold-water soluble high M_r material (>3 kDa) was obtained from 500 mL drip brew of light, medium or dark roasted Arabica (Colombia) coffee, respectively corresponding to approximately 30% of the dry matter content. Independent from the degree of roast, the highest yields were found in the fractions of >100 and 3–10 kDa (Table 2). Each of these fractions accounted for at least one-third of the cold-water soluble high M_r fraction of the respective coffee brew.

Total carbohydrate contents of the fractions as determined colorimetrically ranged from 28.6 g/100 g to 56.7 g/100 g as detailed in Table 3. Independent from the degree of roast, the 3–10 kDa M_r fractions contained significantly less carbohydrates (28.6–33.7 g/100 g) than the fractions

Table 2. Yields of cold-water soluble high M_r fractions obtained by ultrafiltration from 500 mL drip brewed light, medium and dark roasted Colombia coffee

M_r range (kDa)	Light roasted	Medium roasted (g)	Dark roasted
>100	0.63	0.77	0.80
50–100	0.14	0.25	0.35
10–50	0.21	0.55	0.15
3–10	0.93	0.76	0.93

Table 3. Total carbohydrate contents and monosaccharide compositions of cold-water soluble high M_r fractions obtained from drip brewed Colombia coffees

Roasting degree	M_r range (kDa)	Total sugar[a] (g/100 g)	Total sugar[b] (g/100g)	Rhamnose[a]	Arabinose[a]	Mannose[a]	Glucose[a]	Galactose[a] (mol%)
Light	>100	49.6 ± 0.6	49.6 ± 1.5	4.8 ± 0.2	21.5 ± 0.1	15.8 ± 0.3	1.4 ± 0.4	56.5 ± 0.6
	50–100	37.3 ± 0.8	40.6 ± 0.6	3.3 ± 0.2	17.8 ± 0.2	19.6 ± 0.0	1.7 ± 0.5	57.6 ± 0.4
	10–50	38.0 ± 3.7	41.8 ± 1.0	2.3 ± 0.3	13.8 ± 0.6	37.7 ± 0.1	1.2 ± 0.1	45.0 ± 0.4
	3–10	23.3 ± 0.6	28.6 ± 0.7	2.8 ± 0.3	21.1 ± 0.2	27.2 ± 0.1	2.5 ± 0.3	46.5 ± 0.6
Medium	>100	40.0 ± 0.7	53.7 ± 1.9	4.5 ± 0.1	15.9 ± 0.3	26.7 ± 0.1	3.9 ± 0.1	49.0 ± 0.3
	50–100	39.3 ± 1.1	50.0 ± 2.3	2.3 ± 0.0	11.1 ± 0.0	44.2 ± 0.5	3.1 ± 1.0	39.3 ± 0.5
	10–50	42.7 ± 1.6	56.7 ± 1.4	2.1 ± 0.1	8.4 ± 0.1	57.2 ± 0.0	2.2 ± 0.2	30.1 ± 0.0
	3–10	25.9 ± 0.4	33.2 ± 1.0	3.6 ± 0.0	18.5 ± 0.1	26.9 ± 0.1	4.0 ± 1.1	47.1 ± 1.1
Dark	>100	35.3 ± 0.5	47.0 ± 0.5	3.0 ± 0.0	14.2 ± 0.1	39.8 ± 0.5	0.8 ± 0.1	42.2 ± 0.5
	50–100	36.1 ± 1.3	47.0 ± 1.0	1.4 ± 0.1	13.7 ± 0.2	38.6 ± 0.1	0.6 ± 0.0	45.7 ± 0.4
	10–50	32.8 ± 2.3	42.4 ± 2.0	1.7 ± 0.1	12.1 ± 0.2	46.7 ± 0.1	0.7 ± 0.0	38.8 ± 0.3
	3–10	28.2 ± 1.4	33.7 ± 0.5	2.5 ± 0.2	15.6 ± 0.7	34.7 ± 0.3	2.0 ± 0.2	45.2 ± 0.1

a) Determined by alditol acetate method.
b) Determined by phenol-sulfuric-acid method.

Table 4. Absorbance at 280 nm and 405 nm, reducing activity and antioxidant activity of cold-water soluble high M_r fractions obtained from drip brews of light, medium, and dark roasted Colombia coffees

Roasting degree	M_r range (kDa)	Absorbance at 280 nm (au)	Absorbance at 405 nm (au)	RE	AOXP values	TE
Light	>100	0.347 ± 0.011	0.132 ± 0.004	1.42 ± 0.04	1.31 ± 0.04	0.160 ± 0.021
	50–100	0.510 ± 0.011	0.177 ± 0.006	2.10 ± 0.05	1.79 ± 0.03	0.231 ± 0.020
	10–50	0.540 ± 0.005	0.185 ± 0.003	2.14 ± 0.06	1.98 ± 0.07	0.250 ± 0.022
	3–10	0.703 ± 0.003	0.167 ± 0.010	2.62 ± 0.05	3.06 ± 0.17	0.337 ± 0.023
Medium	>100	0.559 ± 0.005	0.204 ± 0.002	1.87 ± 0.02	2.01 ± 0.10	0.194 ± 0.006
	50–100	0.638 ± 0.004	0.228 ± 0.008	2.04 ± 0.04	1.78 ± 0.08	0.234 ± 0.008
	10–50	0.579 ± 0.007	0.201 ± 0.001	1.80 ± 0.04	1.42 ± 0.02	0.198 ± 0.011
	3–10	0.806 ± 0.015	0.207 ± 0.003	2.85 ± 0.09	2.34 ± 0.04	0.316 ± 0.010
Dark	>100	0.582 ± 0.007	0.206 ± 0.005	1.91 ± 0.03	2.66 ± 0.04	0.217 ± 0.010
	50–100	0.536 ± 0.004	0.175 ± 0.004	1.76 ± 0.06	1.74 ± 0.01	0.203 ± 0.018
	10–50	0.634 ± 0.025	0.201 ± 0.011	1.97 ± 0.06	1.83 ± 0.02	0.242 ± 0.016
	3–10	0.658 ± 0.003	0.220 ± 0.003	2.72 ± 0.06	2.03 ± 0.06	0.331 ± 0.022

>10 kDa (40.6–56.7 g/100 g). This indicates the presence of higher amounts of unknown substances such as Maillard reaction products in the 3–10 kDa fractions. Although these fractions also showed the highest absorbance at 280 nm (Table 4), the absorbance at 405 nm (Table 4) was comparable to those of fractions >10 kDa. Total carbohydrate contents as determined by the sum of alditol acetates after hydrolysis and derivatization were generally slightly lower (23.3–49.6 g/100 g) than those obtained by the colorimetrical phenol–sulfuric acid method (Table 3). This may partly be due to noncarbohydrate substances which interfered with the colorimetric assay: the smallest differences in the carbohydrate contents between the two methodologies were found for the light roasted coffee fractions. However, results from both methods should be considered for characterizing these samples, since incorporation of carbohydrate units into melanoidins *via* linkages different from glycosidic linkages may prevent monosaccharide liberation during hydrolysis leading to an underestimation of total carbohydrate contents when using the alditol acetate methodology.

Mannose, galactose, and arabinose were the major carbohydrate units which derived from the two major polysaccharides of coffee brews: galactomannans and arabinogalactans (Table 3), both being present in all high M_r fractions. Cold-water soluble fractions obtained from the light roasted coffee brew showed higher arabinogalactan/galactomannan ratios (1.4–4.0) than cold-water soluble fractions from medium or dark roasted coffee infusions (0.5–2.0 and 0.9–1.3, respectively) (Table 5). This is in agreement with results

Table 5. Glycosidic linkage composition (mol%) of polysaccharides in cold-water soluble high M_r fractions obtained from drip-brewed Colombia coffees as determined by methylation analysis

Glycosidically linked monosaccharide residues[a)]	Light roasted				Medium roasted				Dark roasted			
	<100	50–100	10–50	3–10	>100	50–100	10–50	3–10	>100	50–100	10–50	3–10
							kDa					
T-Rha*p*	6.1	3.1	1.4	3.0	4.6	1.5	1.0	2.9	3.3	1.9	1.0	2.7
T-Ara*f*	15.4	17.0	12.6	16.1	12.4	9.2	6.7	14.6	8.5	8.5	8.5	10.9
5-Ara*f*	8.0	6.3	3.0	6.1	5.9	2.6	2.1	5.6	4.2	3.3	2.4	4.6
T-Gal*p*	9.4	9.5	9.0	9.4	9.3	7.9	6.4	9.9	9.0	9.6	8.9	10.3
3-Gal*p*	21.5	20.3	17.4	15.2	19.1	16.4	10.3	16.1	16.5	17.3	14.3	14.8
6-Gal*p*	5.6	5.9	4.3	5.9	4.8	3.4	3.1	6.1	4.5	5.2	4.2	5.5
3,6-Gal*p*	16.4	15.4	11.9	10.9	13.1	10.5	6.9	12.1	10.0	11.5	9.9	9.2
T-Man*p*	1.0	1.7	3.4	3.3	2.0	3.1	5.1	3.0	3.4	3.1	3.7	4.1
4-Man*p*	14.5	15.3	34.1	26.7	26.3	42.0	54.3	27.1	37.6	36.1	43.9	34.3
4,6-Man*p*	1.5	1.4	2.0	1.7	1.7	2.4	3.0	1.5	2.0	2.7	2.4	1.8
T-Glc*p*	0.1	0.4	0.2	0.8	0.2	0.1	0.2	0.5	0.2	0.2	0.2	0.4
4-Glc*p*	0.4	3.7	0.7	0.8	0.8	0.7	0.8	0.7	0.9	0.7	0.6	1.3
Arabinogalactan/Galactomannan	4.0	3.7	1.4	1.9	2.0	1.0	0.5	1.9	1.1	1.2	0.9	1.3
Total Man*p*/4,6-Man*p*	11.1	13.2	20.1	18.6	17.4	19.6	20.8	21.8	21.3	15.6	20.6	22.6
Gal[b)]/Ara	2.2	2.1	2.6	1.8	2.4	3.0	2.7	2.1	3.0	3.5	3.2	2.5
3-Gal*p*/6-Gal*p*	3.8	3.5	4.0	2.6	4.0	4.8	3.3	2.6	3.7	3.3	3.4	2.7
(3-Gal*p* + 6-Gal*p*)/3,6-Gal*p*	1.7	1.7	1.8	1.9	1.8	1.9	1.9	1.8	2.1	2.0	1.9	2.2
T-Ara*f*/5-Ara*f*	1.9	2.7	4.2	2.7	2.1	3.5	3.3	2.6	2.0	2.6	3.5	2.3
T-Ara*f*/3,6-Gal*p*	0.9	1.1	1.1	1.5	0.9	0.9	1.0	1.2	0.9	0.7	0.9	1.2

a) Key to abbreviations of glycosidically linked monosaccharide residues: T-, terminally linked; 5-, (1 → 5)-linked; 3-, (1 → 3)-linked; 6-, (1 → 6)-linked; 3,6-, (1 → 3,6)-linked; 4-, (1 → 4)-linked; 4,6-, (1 → 4,6)-linked; Rha, rhamnose; Ara, arabinose; Gal, galactose; Man, mannose, Glc, glucose; *p*, pyranosyl residue; *f*, furanosyl residue.

b) Galactosyl residues attributed to galactomannans (equivalent to molar ratio of 4,6-Man*p*) not included.

of Redgwell *et al.*, who observed that the extractability of mannans is enhanced with increase in degree of roast [7, 17]. Lower total Man*p*/4,6-Man*p* ratios were found in fractions obtained from light roasted coffee than from medium or dark roasted coffee (11.1–20.1 < 17.4–21.8 ~ 15.6–22.6, Table 5), indicating that galactomannans extracted from light roasted coffee were more substituted. The increasing degree of roast was also accompanied by an increase in the galactose/arabinose ratio (1.8–2.6 > 2.1–3.0 > 2.5–3.5, Table 5) and the (3-Gal*p* + 6-Gal*p*)/3,6-Gal*p* ratio (1.7–1.9 < 1.8–1.9 < 1.9–2.2, Table 5) of arabinogalactans. This is due to roasting-induced degradation of arabinogalactans which most strongly affects arabinosyl side chains, resulting in lower branching degrees and galactose/arabinose ratios [17]. With increase in degree of roast the arabinose proportions decreased independently from the M_r fraction investigated. Moreover, within the same coffee brews arabinogalactans with the highest arabinose proportions were found in the fractions with the lowest M_rs.

Absorbance at 405 nm was measured as a parameter to estimate differences in brown color (Table 4). For each type of coffee only slight differences were measured for the different M_r fractions. This was also confirmed by color dilution analysis [9] of the different M_r fractions obtained from the medium roasted coffee. For all four high M_r ranges a color dilution factor of 7 was determined (starting concentration of 1 mg/mL). However, high M_r fractions from light roasted coffee showed lower absorbance values at 405 nm (0.132–0.185 au) than fractions from medium roasted coffee (0.201–0.228 au), but no further increase was measured for high M_r fractions of dark roasted coffee (0.175–0.220 au).

As already indicated by the absorbance at 280 nm, 3–10 kDa fractions had stronger reducing properties (2.62–2.85 RE) than fractions > 10 kDa (1.42–2.14 RE) (Table 4). Similar trends were observed for the antioxidant activity measured by the TEAC assay as described by Re *et al*. [13]. The results obtained with the leucomethylene blue assay [11] differed somewhat (Table 4). This is in accordance with different mechanisms underlying these antioxidant assays. Whereas the TEAC assay is supposed to predominantly measure the reducing abilities of antioxidant compounds [18], the leucomethylene blue test considers both the reducing and the radical scavenging properties [11]. Using the TEAC-assay, no significant differences in the antioxidant activities were observed for the fractions 3–10 kDa from differently roasted coffees. The antioxidant activities of the 3–10 kDa fractions as determined by the leucomethylene methodology, by contrast, decreased with increase in degree of roast (3.06 > 2.34 > 2.03 AOXP values). This indicates that "antioxidant" components of this fraction were either destroyed or degraded resulting in frag-

ments < 3 kDa or, more likely, participated in further reactions such as crosslinking or polymerization reactions, leading to substances with a higher M_r. However, all three methods showed that the antioxidant activity of fractions > 100 kDa increased with the degree of roast. To our knowledge, the effect of roasting on the antioxidant activity of coffee fractions of different high M_r ranges prepared by ultrafiltration has not been reported previously. Roasting-induced changes of antioxidant activity were analyzed for whole coffee brews, fractions obtained by gel filtration chromatography using Sephadex G25, or a high M_r fraction obtained by ultrafiltration (> 10 kDa) [19 – 22]. For example, Borrelli *et al*. [22] showed that the antioxidant activity of high M_r coffee melanoidins (obtained by Sephadex G25 chromatography) decreased with increase in degree of roast if determined by TEAC assay, but the ability to prevent linoleic acid peroxidation increased with increase in degree of roast. However, due to the different preparation procedures of the high M_r fractions a direct comparison with our results is not possible.

3.2 Fermentability of high M_r coffee fractions by human fecal microbiota

The *in vitro* fermentability of coffee polysaccharides was recently demonstrated using soluble coffee dietary fiber as a substrate [1]. The preparation of coffee dietary fiber included the required ethanol precipitation step, thus it is expected to contain polysaccharides widely varying in M_r. Chemical characterization of the ultrafiltration fractions used here (3.1) showed that arabinogalactans and galactomannans were present in all M_r fractions investigated (3 – 10, 10 – 50, 50 – 100, and > 100 kDa). Therefore these coffee fractions provide additional information about the degradability of arabinogalactans and galactomannans differing in M_r. In contrast to the fiber fraction, the M_r fractions studied here include polysaccharides which are ethanol soluble as well. In addition, these fractions include all high M_r nonpolysaccharide compounds such as melanoidins regardless their behavior during ethanol precipitation. The fractions used in the present study also provide information about a possible effect of the degree of roast on the fermentability of coffee polysaccharides.

To investigate the fermentability of the described high M_r coffee fractions, each fraction was added to batch cultures with ten-fold diluted fecal bacteria and incubated anaerobically at 37°C. For comparison, glucose instead of coffee fractions was used in control experiments. The OD at 600 nm, which reflects bacterial cell density, increased over time showing that the high M_r fractions served as a growth substrate for the colonic microbiota (data not shown). Whereas the OD of the fecal bacterial suspensions supplemented with glucose was maximal at 6 h, the OD of the fecal bacteria supplemented with the different coffee fractions increased more slowly during 24 h without reaching a maximum. The slower increase in OD of the coffee samples reflects the complex structures of the high M_r coffee fractions which are more difficult to break down. As the OD does not provide information on cell numbers, FISH followed by microscopic cell enumeration was performed for all samples collected at 0 and 24 h (Fig. 1). The differences found in the total cell counts after 24 h for the fractions > 100, 50 – 100, 10 – 50 kDa, and the different degrees of roast were not significant. For the 3 – 10 kDa fraction significantly lower cell numbers were obtained for the light and the dark roasted coffee compared to the corresponding M_r fraction from the medium roasted coffee. This can be explained by the generally lower carbohydrate contents of the 3 – 10 kDa fractions (Table 3).

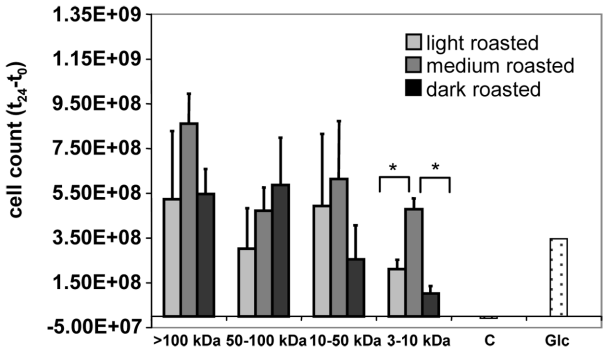

Figure 1. Changes in total cell counts after 24 h of fermentation of different coffee fractions with human fecal bacteria. The coffee fractions represent different M_r ranges and were obtained from three coffees differing in their degree of roast. C, control (without substrate); Glc, 10 mM glucose; *p< 0.05.

3.3 Utilization of polysaccharides from high M_r fractions by human fecal microbiota

After 24 h of fermentation polysaccharides were extensively degraded in all coffee fractions (by 84.3 – 95.6 and 75.3 – 90.3% as determined with the alditol acetate and phenol – sulfuric acid method, respectively) with no distinct effects of the degree of roast (Table 6). The proportion of carbohydrates degraded from the soluble fiber fraction from medium roasted coffee brew was reported to be in the same range (85 and 91%, respectively) [1]. It may be concluded that higher amounts of melanoidins, as present in coffee fractions obtained by stepwise ultrafiltration, do not negatively affect the utilization of the polysaccharides being present in these fractions. However, the soluble fiber fraction from coffee also contained unknown, chromophoric compounds and it cannot be ruled out that Maillard reaction products, which are partly covalently linked to coffee polysaccharides [23, 24], have some influence on their degradability.

Intestinal bacteria utilized arabinogalactans to a smaller extent than galactomannans (Table 6), which was also

Table 6. Degradation of carbohydrates and decrease in absorbance at 405 nm and 280 nm after 24 h of fermentation as compared to the starting material

Roasting degree	M_r range (kDa)	Total carbohydrate degradation[a] (%)	Total carbohydrate degradation[b] (%)	Arabinose degradation[b] (%)	Galactose degradation[b] (%)	Mannose degradation[b] (%)	Decrease of absorbance at 405 nm (%)	Decrease of absorbance at 280 nm (%)
Light	>100	81.2	88.2	80.3	89.9	95.6	21.7	13.4
	50–100	84.5	91.7	86.6	92.6	96.6	22.2	14.8
	10–50	80.4	90.3	81.0	89.2	97.0	27.1	17.8
	3–10	80.1	91.5	88.0	91.5	97.4	29.0	17.9
Medium	>100	79.9	86.5	74.8	86.9	92.8	10.6	5.1
	50–100	82.1	93.3	76.1	92.8	98.2	6.9	5.3
	10–50	90.3	95.6	87.9	93.0	98.8	7.9	6.1
	3–10	77.4	91.2	85.9	91.3	97.0	5.4	2.5
Dark	>100	75.3	84.3	70.1	80.2	95.0	9.0	6.2
	50–100	80.5	88.8	80.2	86.7	95.8	16.9	10.7
	10–50	82.1	90.7	81.4	87.7	97.1	18.1	12.8
	3–10	79.7	91.0	84.2	90.3	97.6	20.4	5.6

a) Determined by phenol–sulfuric-acid method.
b) Determined by alditol acetate method.

recently shown for soluble dietary fiber polysaccharides from coffee [1]. This was mainly due to a weaker degradation of arabinosyl (70.1–88.0% decrease) and galactosyl residues (80.2–93.0% decrease) as compared to mannosyl residues, which were most extensively utilized (92.8–98.8% degradation). Owing to the low substitution degree of coffee galactomannans the galactose residues mainly stem from arabinogalactans. No distinct differences were noticed for the degradation of mannosyl residues between different fractions. In contrast, degradability of arabinosyl residues and galactosyl residues was somewhat variable, reflecting a higher complexity and heterogeneity of roasted coffee arabinogalactans. Considering all M_r fractions total degradation of arabinosyl and galactosyl residues after 24 h of fermentation could neither be strongly correlated with the M_r range nor with structural characteristics as determined by methylation analysis (Table 5).

Fermentations were repeated on a larger scale using the M_r fractions of a medium roasted coffee brew as substrates to follow carbohydrate degradation by fecal bacteria over time in more detail. The degradation of mannosyl residues was shown to be independent from the M_r range, whereas the degradation rates of galactosyl and arabinosyl residues varied for the different M_r fractions (Fig. 2). Galactose degradation rates increased in the sequence >100, <50–100, ~3–10, and <10–50 kDa while arabinose degradation rates increased in the sequence >100, <3–10, <50–100, and <10–50 kDa. The changes in the polysaccharide structure observed here were similar to those recently reported for the fermentation of soluble coffee fiber by human fecal microbiota [1], again indicating that higher amounts of non-carbohydrate substances, as present in high M_r coffee fractions obtained by ultrafiltration, do not affect the carbohydrate utilization.

3.4 Utilization of UV absorbing and brown components from high M_r coffee fractions and changes in antioxidant properties

Apart from polysaccharides, chemically noncharacterized brown and UV-active components, *e.g.*, Maillard reaction products, were modified and hence possibly utilized by colonic microbiota as indicated by decreases of up to 29 and 18% in the absorbance values at 405 and 280 nm, respectively after 24 h of fermentation (Table 6). Additional information was obtained from the large-scale fermentations of the medium roasted coffee fractions. Samples fermented for 0, 3, 6, 12, and 24 h were fractionated into low and high M_r material *via* ultracentrifugation (MWCO 1 kDa). The absorbance at 405 and 280 nm indicated that no significant amounts of brown colored or UV-active low M_r substances (<1 kDa) were released during fermentation (data not shown). It may be concluded that the M_rs of the chromophores were still >1 kDa after fermentation and/or they were still bound to uncolored high M_r substances, such as residual oligosaccharides or proteins. Since the low M_r components of the fermentation media were expected to interfere with the antioxidant assays, antioxidant, and reducing activities of the fermented samples were only measured in the retentates >1 kDa. However, since no significant amounts of low M_r UV-active substances were released during fermentation it can be assumed that the remaining antioxidants were mainly present in the retentates. Figure 3 shows exemplarily the time dependent changes of reducing/antioxidant properties of the fraction >100 kDa as measured with the ferricyanide method, the TEAC assay and the leucomethylene blue method. The same trends were observed for the fractions <100 kDa (data not shown). In all three assays the 0-h samples already showed a significantly reduced antioxidant activity when

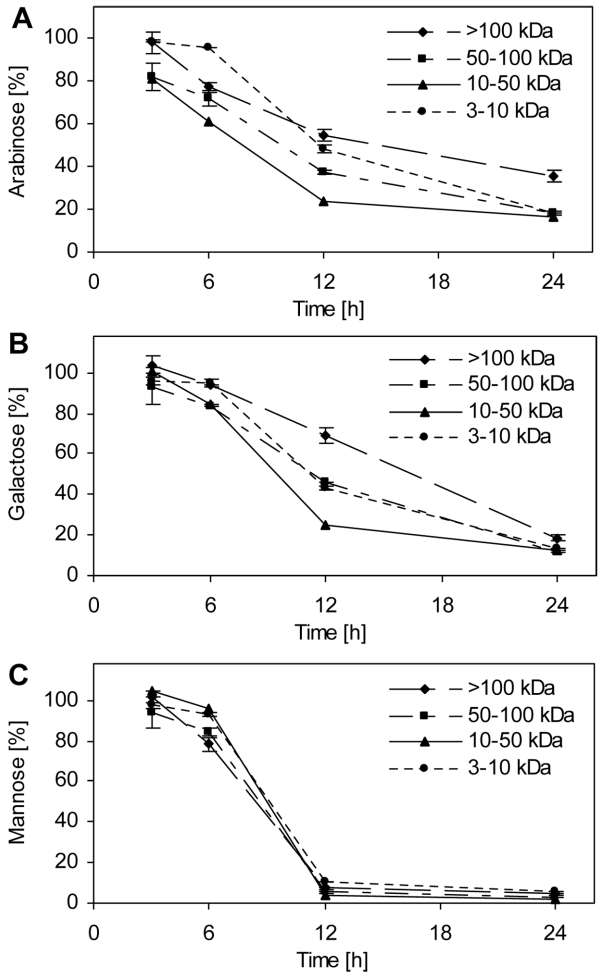

Figure 2. Degradation of arabinosyl (A), galactosyl (B), and mannosyl (C) residues of polysaccharides from high M_r coffee fractions during fermentation with a human fecal sample. Results refer to the amount of the respective monosaccharide residue determined at 0 h which was set to 100%.

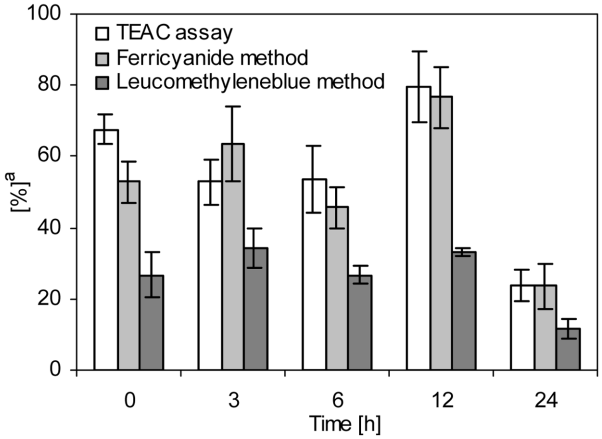

Figure 3. Changes in reducing and antioxidant activities of a high M_r coffee fraction (>100 kDa; medium roasted coffee) during fermentation with a human fecal sample. (a) Results refer to the activity of the original fraction which was set to 100%.

compared to the original fraction. This was possibly due to oxidation, loss of active compounds in the 0-h samples during ultracentrifugation or by reaction with components of the fermentation medium. However, after 24 h of fermentation the antioxidant activity of the fermented material decreased even further. Since it is unlikely that it would take more than 12 h at room temperature for a reaction between medium compounds and the substrate to start, we conclude that a further reduction of AA caused by reaction with medium compounds would have occurred already between 0 and 12 h. Therefore the decrease only after 24 h indicates the utilization and/or inactivation of antioxidant components by gut bacteria. Nevertheless, even after 24 h of fermentation the remainder still showed antioxidant properties *in vitro*. It can be concluded that, even after being partially degraded and/or modified by intestinal bacteria, high M_r coffee components may act as scavengers for reactive oxygen species, which are thought to have a promoting effect on colon cancer [4, 25]. In light of these findings, the observed slow decrease in antioxidant activity by intestinal bacteria may be considered favorable for human health. However, the fate of high M_r coffee antioxidants during intestinal transit remains to be investigated *in vivo*.

3.5 Production of SCFA from high M_r coffee fractions by human fecal microbiota

Acetate, propionate, and butyrate were detected as bacterial fermentation products (Table 7). After 6 and 12 h of fermentation the net total SCFA produced from glucose (for comparison) were significantly higher than those formed from coffee fractions. After 24 h total SCFA formed from glucose or coffee fractions were comparable. Only the fractions >100 and 10–50 kDa from the light roasted coffee led to significantly higher amounts of SCFA. The kinetics of acetate and butyrate formation within 12 h were similar to those of total SCFA. Significantly lower concentrations of these two acids were measured at 6 and 12 h when compared to glucose. The amounts of acetate produced from the high M_r coffee fractions after 24 h of fermentation were similar or even higher than those produced from glucose. In contrast, the amounts of butyrate formed within 24 h from the coffee fractions were mostly significantly lower than those formed from glucose. Considering the different degrees of roast it is remarkable that fermentation of the light roasted coffee fractions resulted in higher total SCFA production compared to the fractions of the medium and dark roasted coffees, although no differences in total carbohydrate degradation were observed. The fermentation of fractions obtained from light roasted coffee also resulted in slightly higher molar proportions of butyrate (8–12% of

Table 7. SCFA production during fermentation of cold-water soluble high M_r fractions from drip brewed Colombia coffees by human fecal microbiota

(A)

Degree of roast	M_r range (kDa)	Acetate (mM)			Propionate (mM)		
		6 h	12 h	24 h	6 h	12 h	24 h
Light	>100	1.68 ± 0.08	4.49 ± 0.37	9.21 ± 0.23	0.58 ± 0.06	1.44 ± 0.22	3.19 ± 0.17
	50–100	2.26 ± 0.09	4.09 ± 0.20	6.87 ± 0.40	0.90 ± 0.04	2.13 ± 0.15	2.87 ± 0.15
	10–50	0.72 ± 0.34	4.43 ± 0.11	7.30 ± 0.46	0.32 ± 0.06	3.03 ± 0.23	4.49 ± 0.35
	3–10	2.10 ± 0.69	4.11 ± 0.16	6.01 ± 0.49	0.53 ± 0.18	2.36 ± 0.12	2.38 ± 0.17
Medium	>100	2.63 ± 0.11	4.86 ± 0.25	5.44 ± 1.57	1.36 ± 0.07	2.10 ± 0.05	2.45 ± 0.36
	50–100	3.38 ± 0.63	4.19 ± 0.35	3.81 ± 0.32	1.77 ± 0.22	2.47 ± 0.06	2.21 ± 0.28
	10–50[a)]	1.25	5.49	5.06	0.48	2.80	2.26
	3–10	2.52 ± 0.49	4.16 ± 0.38	4.41 ± 0.43	1.73 ± 0.35	2.14 ± 0.22	2.26 ± 0.18
Dark	>100	1.44 ± 0.14	4.29 ± 0.66	5.81 ± 0.50	0.28 ± 0.04	1.57 ± 0.14	2.56 ± 0.29
	50–100	1.77 ± 0.11	5.47 ± 0.46	4.49 ± 0.64	0.34 ± 0.07	2.57 ± 0.37	2.46 ± 0.18
	10–50	1.77 ± 0.20	4.26 ± 0.11	6.11 ± 0.37	0.68 ± 0.07	2.72 ± 0.53	2.64 ± 0.31
	3–10	1.48 ± 0.71	3.25 ± 0.35	6.50 ± 0.44	0.61 ± 0.21	2.02 ± 0.21	2.60 ± 0.34
	glucose	5.93 ± 1.35	7.25 ± 1.27	6.38 ± 1.43	1.50 ± 0.48	2.13 ± 0.30	1.62 ± 0.06

(B)

Degree of roast	M_r range (kDa)	Butyrate (mM)			Total (mM)		
		6 h	12 h	24 h	6 h	12 h	24 h
Light	>100	0.34 ± 0.06	0.88 ± 0.08	1.29 ± 0.11	2.68 ± 0.09	6.61 ± 0.41	13.87 ± 0.37
	50–100	0.46 ± 0.03	1.03 ± 0.04	1.27 ± 0.07	3.67 ± 0.09	7.15 ± 0.36	10.58 ± 0.55
	10–50	0.09 ± 0.10	0.70 ± 0.15	1.21 ± 0.12	1.68 ± 0.46	8.20 ± 0.16	12.93 ± 0.32
	3–10	0.10 ± 0.10	0.57 ± 0.21	0.79 ± 0.09	1.79 ± 0.97	5.38 ± 1.69	8.72 ± 0.66
Medium	>100	0.31 ± 0.01	0.67 ± 0.06	0.77 ± 0.54	4.31 ± 0.17	7.63 ± 0.32	8.67 ± 2.68
	50–100	0.32 ± 0.06	0.58 ± 0.03	0.60 ± 0.07	5.46 ± 0.86	7.24 ± 0.33	6.62 ± 0.66
	10–50[a)]	0.08	0.26	0.20	1.81	8.54	7.91
	3–10	0.15 ± 0.10	0.39 ± 0.10	0.33 ± 0.08	4.31 ± 0.17	6.69 ± 0.67	6.02 ± 0.67
Dark	>100	0.03 ± 0.04	0.13 ± 0.11	0.35 ± 0.03	1.75 ± 0.22	5.99 ± 0.65	8.71 ± 0.81
	50–100	0.06 ± 0.01	0.26 ± 0.02	0.32 ± 0.02	2.18 ± 0.19	8.29 ± 0.82	7.29 ± 0.82
	10–50	0.16 ± 0.03	0.57 ± 0.07	0.64 ± 0.10	2.61 ± 0.17	7.55 ± 0.51	9.38 ± 0.27
	3–10	0.06 ± 0.07	0.42 ± 0.15	0.69 ± 0.07	2.16 ± 1.00	5.70 ± 0.46	9.70 ± 0.37
	glucose	0.86 ± 0.16	1.28 ± 0.28	1.08 ± 0.19	8.28 ± 1.74	10.67 ± 1.46	9.08 ± 1.31

a) Analysis of just one sample.

total SCFA), when compared to fractions from medium and dark roasted coffee brews (3–9 and 4–7%, respectively). The production of SCFA correlates with positive effects on human health [26]. SCFA are metabolized by host tissues and have a trophic effect on the colonic mucosa with butyrate being the preferred energy substrate of the colonocytes. The amount of propionate produced from glucose and the medium roasted coffee fractions were comparable after 6 h of fermentation, while the propionate concentrations resulting from the light and dark roasted coffee fractions were significantly lower at this time. However, they increased to the same level within 12 h as observed for glucose and the medium roasted coffee fractions. After 24 h of fermentation the concentrations of propionate formed from the coffee fractions were, independent from the degree of roast, significantly higher than those formed from glucose. Compared to other nondigestible polysaccharides such as resistant starch or pectin [27], coffee arabinogalactans, and galactomannans lead to higher propionate production. Despite some structural differences, similar observations have been made for arabinogalactans and galactomannans from other sources such as larch and guar [28]. The reported molar ratios of acetate, propionate and butyrate for larch arabinogalactan and guar (50:42:8 and 58:29:13, respectively) were similar to those we determined for the high M_r coffee fractions (56–67:23–35:3–12). The comparatively fast and strong increase in propionate in response to the coffee preparations compared to glucose also indicates that species belonging to the genus *Bacteroides* were preferentially involved in the degradation of the coffee polysaccharides as confirmed by FISH analysis (3.6).

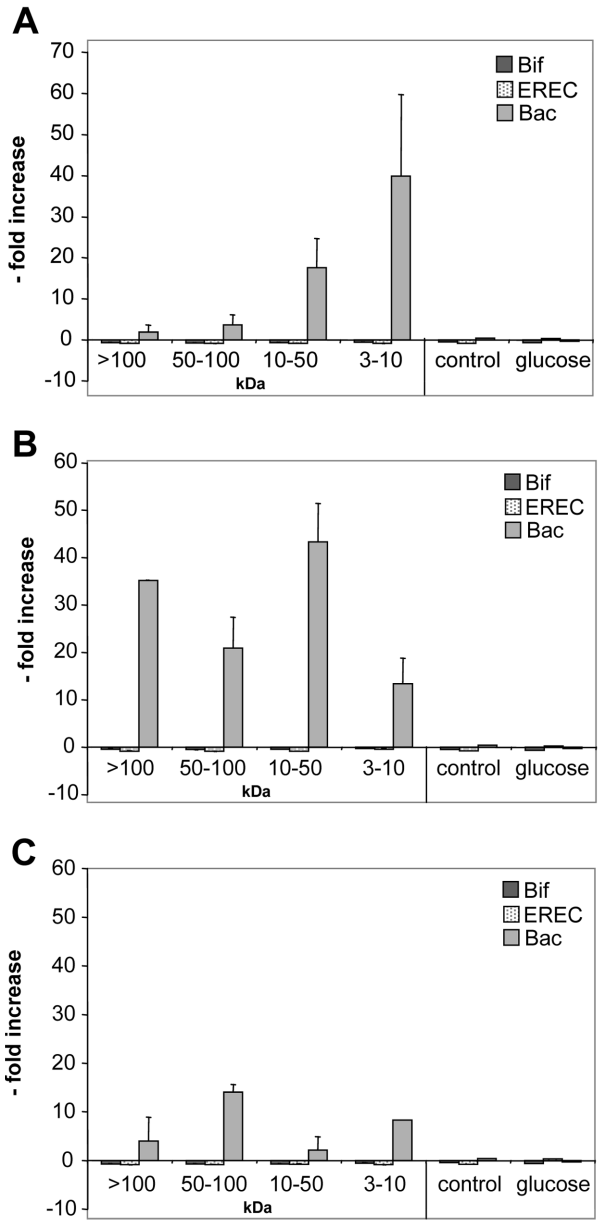

Figure 4. Changes in the proportions of bifidobacteria (Bif), *E. rectale – C. coccoides* cluster (EREC) and *Bacteroides – Prevotella* group compared to the total cell counts after 24 h of fermentation of four different high M_r coffee fractions of three degrees of roast (A, light roasted; B, medium roasted; C, dark roasted) with human fecal microbiota.

3.6 Influence of the fermentation of high M_r coffee fractions on the composition of human fecal microbiota

FISH analysis was carried out to monitor changes in the proportion of dominant bacterial groups during degradation of the different coffee fractions or glucose. The proportions of three dominant bacterial groups (Fig. 4) relative to the total cell counts at 24 h were compared to those at 0 h. Changes in the proportion of bacterial cells belonging to the *Eubacterium rectale – Clostridium coccoides* cluster or to the genus *Bifidobacterium* were minimal (Fig. 4). In contrast, the proportion of the *Bacteroides – Prevotella* group increased in all fermentation experiments with any of the coffee fractions as substrates. The light roasted coffee (Fig. 4A) led to increasing proportions of *Bacteroides – Prevotella* with decrease in M_r of the fraction: The proportion of this bacterial group increased as follows: >100 kDa approximately two-fold, for 50 – 100 kDa approximately four-fold, for 10 – 50 kDa approximately 18-fold, and for 3 – 10 kDa approximately 40-fold within 24 h of incubation. The increase in the *Bacteroides – Prevotella* cell counts of the medium and dark roasted coffee fractions was unrelated to the M_r range of the coffee fractions (Figs. 4B and C). The growth of species belonging to the *Bacteroides – Prevotella* group was previously shown to be strongly stimulated by coffee dietary fiber when incubated with fecal microbiota [1]. *Bacteroides* species are well known for their ability to degrade a large variety of complex carbohydrates because they are equipped with a wide range of carbohydrate-depolymerizing enzymes [29].

Changes in the composition of the microbiota during degradation of the four different M_r coffee fractions obtained from medium roasted coffee were also monitored by PCR-DGGE. DGGE profiles obtained from samples taken at 0 h of fermentation were compared to those taken at 24 h (Fig. 5). At 0 h the DGGE profiles were identical for all samples including the control. However, after 24 h of fermentation the band patterns had changed: without substrate addition the number of bands decreased from 21 bands to 16 bands, whereas the addition of coffee preparations led to an increase to 33 bands independent from the M_r of the coffee fractions. This observation indicates that the growth of some bacterial species was stimulated by the coffee fractions under investigation. Since the observed additional bands were the same for each coffee fraction it can be concluded that the same bacterial species were involved in the degradation of the different coffee fractions. As the same changes in microbial composition were independent from the polysaccharide content of the various coffee fractions (Table 3), it follows that the composition of the intestinal microbiota was mainly affected by the availability of polysaccharides.

4 Concluding remarks

Galactomannans and arabinogalactans are the main polysaccharides in coffee brews and make up between one-fourth to one-half of the high M_r (>3 kDa) coffee fractions depending on the M_r range of the respective fraction. These polysaccharides were extensively fermented by intestinal bacteria, leading to the production of SCFA, which are gen-

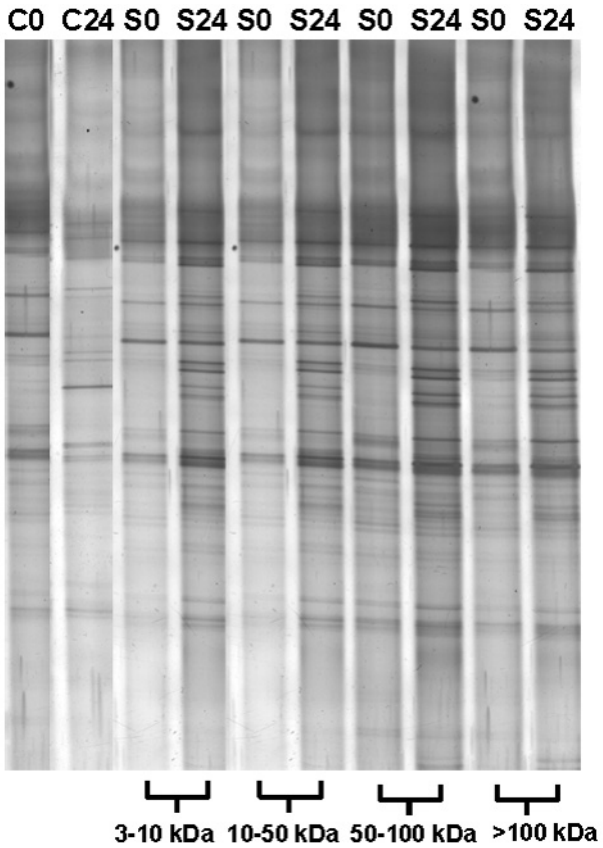

Figure 5. Changes in microbial composition of a human fecal sample during fermentation with high M_r coffee fractions as analyzed by DGGE. C0, control (medium and bacteria without substrate) at 0 h; C24, control at 24 h; S0, sample (medium, bacteria, and the particular coffee fraction) at 0 h; S24, sample at 24 h.

erally known to promote positive physiological effects. Butyrate, which plays a special role, was produced in slightly higher molar proportions from the light roasted coffee fractions than from the medium and dark roasted coffee fractions. Coffee polysaccharides affect the composition of the gut microbiota stimulating preferentially the members of the *Bacteroides–Prevotella* group, which possess a wealth of polysaccharide depolymerizing enzymes [29]. Hence, polysaccharides were presumably the preferred substrates whereas the fermentation of noncarbohydrate material such as Maillard reaction products, apparently played a minor role.

Owing to their *in vitro* antioxidant properties, noncarbohydrate high M_r components of coffee brews may also promote positive health effects in view of the discussed role of oxygen radicals in colon carcinogenesis [4]. The antioxidant capacity of the different coffee fractions was fairly stable within the first 12 h of fermentation and only decreased significantly after 24 h of fermentation thus supporting a role of high M_r coffee fractions in the protection against radical stress in the intestine. Surprisingly, low M_r (<1 kDa) UV-active or brown colored material was not generated by polysaccharide degradation. This means that either binding of low-M_r, noncarbohydrate, UV-active, or chromophoric groups hinders the microbial enzymes from degrading carbohydrate compounds close to the linkage points of these noncarbohydrate compounds or that these compounds are of high M_r themselves. Alternatively, the linkage to proteins may prevent or slow down their degradation by gut bacteria. However, the structures of these high M_r antioxidants from coffee beverages and their physiological effects *in vivo* require further investigations.

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The authors have declared no conflict of interest.

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